

# Genetic Mapping and Marker-Assisted Selection for Late Leaf Spot Resistance in *Arachis hypogaea* L.

A.F. Binta<sup>1</sup>, V.I. Oliver<sup>1</sup>, A.Y. Abubakar<sup>2</sup>, I.S. Nasarawa<sup>3</sup>, T.S. Alhaji<sup>4</sup>, T. Mustapha<sup>5</sup>, A.U. Ibrahim<sup>6</sup>, J.I. Mangwa<sup>6</sup>, A.M. Zakari<sup>7</sup>, M.M. Ibrahim<sup>7</sup>, S. Abdullaziz<sup>6,7\*</sup>



<sup>1</sup>Ahmadu Bello University Zaria, Kaduna, Nigeria

<sup>2</sup>Bioresources Development Centre, National Biotechnology Development Agency, Kano, Nigeria

<sup>3</sup>Federal University Dutsin-ma Katsina

<sup>4</sup>Usman Danfodio University Sokoto, Nigeria

<sup>5</sup>Federal University Dutse Jigawa, Nigeria

<sup>6</sup>Nasarawa State University Keffi, Nigeria,

<sup>7</sup>Fujian Agriculture and Forestry University Fuzhou, China



**ABSTRACT:** Late leaf spot (LLS), caused by *Nothopassalora personata*, poses a significant threat to global groundnut (*Arachis hypogaea*) production, causing severe yield losses and increased reliance on costly fungicides. This study focuses on improving LLS resistance in groundnut genotypes in northern Nigeria through genetic mapping and marker-assisted selection (MAS). Twenty diverse genotypes, including local landraces and improved varieties, were evaluated for LLS resistance and pod yield across three Nigerian States. Disease severity was scored on a 1-9 scale, and SSR and SNP markers provided genotypic data. QTL mapping identified three significant QTLs on Chromosomes 2, 5, and 9, with the major QTL, *qLLS1*, explaining 38% of the phenotypic variance. High broad-sense heritability estimates for LLS severity (0.72) and pod yield (0.68) indicate strong genetic control. A significant but negative correlation ( $r = -0.65$ ,  $p < 0.01$ ) between LLS severity and pod yield underscores the potential yield benefits of resistance breeding. The identified QTLs, especially *qLLS1*, provide valuable markers for MAS, facilitating the efficient selection of resistant genotypes. These findings support the development of LLS-resistant cultivars, promoting sustainable groundnut production and food security.

**KEYWORDS:** Disease, Genetic mapping, Late leaf spot, MAS, Quantitative trait loci.

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## I. INTRODUCTION

Groundnut (*Arachis hypogaea* L.), commonly known as peanut, is a globally important legume, valued for its high oil content and protein-rich seeds. It plays a crucial role in food security, economic stability, and rural livelihoods, especially in semi-arid and tropical regions. In Nigeria, groundnut is a vital agricultural commodity contributing significantly to household incomes and rural economies. For instance, in Taraba State, the crop yields a gross margin of ₦90,070 per hectare, with a benefit-cost ratio of 1.44, highlighting its profitability despite production costs of ₦170,610 per hectare (Abdullaziz *et al.*, 2024; Votapwa *et al.*, 2024a, 2024b; Akintunde *et al.*, 2023). However, groundnut production faces considerable challenges from both biotic and abiotic stresses, with late leaf spot (LLS), caused by the fungal pathogen *Nothopassalora personata*, emerging as one of the most severe threats. LLS can cause yield losses exceeding 50% in heavily infested fields, reducing pod

quality and increasing production costs due to reliance on fungicide applications, which also raise environmental concerns (Votapwa *et al.*, 2025; Tukur *et al.*, 2024; Kamalasundari *et al.*, 2024).

Breeding for LLS resistance offers an environmentally friendly and cost-effective alternative to chemical control. However, traditional breeding approaches are limited by the complex inheritance of resistance traits and the significant influence of environmental factors on disease expression (Aziz and Masmoudi, 2024; Manzoor *et al.*, 2024). LLS resistance is polygenic, involving multiple quantitative trait loci (QTLs) with small to moderate effects. Recent advancements have identified 11 QTLs associated with LLS resistance, with one major QTL (*qLLS.LG02*) explaining up to 31% of phenotypic variation (Chen *et al.*, 2024). Additionally, over 160 nucleotide-binding site leucine-rich repeat (NBS-LRR) genes within these QTL regions provide a rich genetic resource for breeding programmes (Chen *et al.*, 2024).

\*Corresponding author: sheiduabdullaziz8@gmail.com

Marker-assisted selection (MAS) has revolutionized groundnut breeding by enabling precise selection of resistant genotypes using molecular markers, significantly reducing the time and resources required for phenotypic screening (Danso *et al.*, 2024; Purkaystha *et al.*, 2024). This strategy has already facilitated the development of advanced breeding lines and commercial varieties with enhanced LLS resistance (Pandey *et al.*, 2024). Nevertheless, challenges remain, including the potential for resistance breakdown due to evolving pathogen populations and the need for continuous refinement of MAS strategies to ensure robust performance across diverse agro-climatic conditions (Zhu *et al.*, 2023). Integrating genomic selection with MAS could further improve breeding efficiency for quantitative disease resistance (Kumari *et al.*, 2024).

While QTLs linked to LLS resistance have been identified in groundnut varieties from other regions, the genetic architecture of resistance in Nigerian groundnut genotypes remains underexplored. Given Nigeria's diverse agro-climatic zones, understanding the genetic factors underlying LLS resistance in local varieties is crucial for developing resilient cultivars adapted to these conditions. This study aims to bridge this knowledge gap by conducting genetic mapping of LLS resistance in a diverse panel of Nigerian groundnut genotypes. Specifically, it focuses on assessing phenotypic variation in LLS resistance and pod yield across different agroecological zones, identifying QTLs associated with LLS resistance using SSR and SNP markers, and evaluating their potential for MAS in breeding programmes. The outcomes will contribute to the development of LLS-resistant groundnut cultivars, enhancing productivity, and promote sustainable agricultural practices in Nigeria.

## II. MATERIALS AND METHODS

### A. Study Area

The study was conducted across three major groundnut-producing states in Nigeria—Nasarawa, Kaduna, and Kano—to capture the diverse environmental conditions affecting groundnut production. These states are characterised by varying climatic conditions, including arid and semi-arid zones, which provided a suitable environment for evaluating disease pressure and groundnut performance. The following locations were used for evaluation during the 2021/2022 growing season:

- Nasarawa State: Semi-arid region with an average rainfall of 500 mm/year, and temperatures ranging from 25°C to 35°C.
- Kaduna State: Semi-arid region with an average rainfall of 600 mm/year, and temperatures ranging from 28°C to 38°C.
- Kano State: Arid region with an average rainfall of 450 mm/year, and temperatures ranging from 30°C to 40°C.

Field trials were conducted at the Institute for Agricultural Research (IAR) Samaru, Zaria, NSUK Teaching and Research Farm in Lafia, and BUK Teaching and Research Farm, offering a diverse range of agro-ecological zones for evaluating the performance of groundnut genotypes under varying environmental conditions.

### B. Plant Materials

A total of 20 groundnut genotypes were used (Table S1), representing a diverse panel that included the Local landraces Dan Hassan (G1), Dan-ja (G3) that are historically cultivated in Nigeria for their potential natural resistance traits (Naidu *et al.*, 2023). Secondly, the improved varieties Samnnut 26 (V1), Samnut 25 (V2) genotypes developed by the Institute for Agricultural Research (IAR), known for higher yield potential, were evaluated. Thirdly, the exotic lines Dansa (E1), which are introduced genotypes with documented resistance to late leaf spots in other regions, were also tested (Achola *et al.*, 2023). This selection aimed to capture a broad genetic base, maximizing the likelihood of identifying significant QTLs for late leaf spot resistance (Bangaru *et al.*, 2023).

### C. Phenotypic Evaluation.

The field trials were conducted in a randomized complete block design (RCBD) with three replicates per genotype. Each plot consisted of five rows, and data collection were carried out on the central rows to minimize border effects (Banla *et al.*, 2020).

### D. Late Leaf Spot Scoring and Pod Yield Measurement

Disease severity was assessed at 60, 75, and 90 days after planting using a 1-9 scale, where 1 indicated no symptoms, 9 represented severe defoliation, and scores were recorded for each plot (Mohammed *et al.*, 2018). At maturity, pod yields were measured for each plot to evaluate the impact of late leaf spots on productivity.

### E. Marker Selection and Genotyping

A combination of Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs) were selected for genotyping (Dwiningsih *et al.*, 2020). Markers linked to disease resistance in previous studies were prioritized. SSR markers were amplified using polymerase chain reaction (PCR), while SNP genotyping was performed using Kompetitive allele-specific PCR (KASP) technology. The resulting data provided a comprehensive genotypic profile for each genotype (Turaki *et al.*, 2017).

### F. Genetic Mapping and QTL Analysis

A genetic linkage map was constructed based on the segregation of SSR and SNP markers across the population (Chen *et al.*, 2024). JoinMap software was used for mapping, with a minimum LOD score of 3 sets for marker grouping. Composite Interval Mapping (CIM) was employed to

identify genomic regions associated with late leaf spot resistance (Zhang, 2020). Significant QTLs were determined using a logarithm of the odds (LOD) score threshold of 3.0, with higher scores indicating stronger associations with the trait of interest.

### G. Statistical Analysis

Analysis of Variance (ANOVA) was conducted to evaluate the differences in late leaf spot severity and pod yield among genotypes, and the interaction between genotype and environment was assessed. Broad-sense heritability for late leaf spot resistance was calculated using Heritability Estimates to determine the proportion of phenotypic variance attributable to genetic factors. Correlation Analysis was carried out to determine the relationship between late leaf spot severity and pod yield. Significant correlations were identified using a threshold of  $p < 0.05$ .

## III. RESULTS AND DISCUSSIONS

The groundnut genotypes exhibited significant variation in Late Leaf Spot (LLS) severity and pod yield across the three study locations, highlighting differences in both disease resistance and productivity under field conditions (Table 1). The disease severity scores among the genotypes ranged from 2.5 to 8.0, reflecting a broad spectrum of susceptibility to LLS. Specifically, the local genotype G3 and the improved variety V2 demonstrated the highest resistance to LLS, with severity scores of 2.5 and 3.0, respectively. In contrast, the exotic genotype E1 was the most susceptible, exhibiting a score of 8.0, which indicates severe disease symptoms.

In addition to disease resistance, pod yield also varied considerably across genotypes, suggesting that genetic differences influenced yield performance under disease pressure (Fig. 1). Notably, genotypes exhibiting higher LLS resistance, such as G3 and V2, maintained higher

pod yields compared to more susceptible varieties. This trend underscores the ability of resistant genotypes to sustain productivity, even in environments prone to late leaf spot outbreaks.

These findings suggest that incorporating LLS-resistant genotypes into breeding programmes could enhance yield stability and disease resilience in groundnut cultivation across diverse Nigerian agroecological zones. This aligns with recent studies by Choudhary *et al.* (2021), who found that groundnut genotypes with higher resistance to diseases tend to maintain productivity even under pathogen pressure. For instance, G3 (local) and V2 (improved) exhibited low disease severity scores (2.5 and 3.0) and minimal yield reductions, demonstrating their resilience to LLS, as similarly observed in other studies (Chen *et al.*, 2024).

The negative impact of high disease severity on pod yield, as seen in the E1 genotype, is consistent with the findings of Kumar *et al.* (2023), who reported significant yield losses in legumes associated with fungal diseases. As highlighted by Savithramma *et al.* (2023), disease severity can lead to yield reductions ranging from 0–90%, depending on the genotype's resistance level. Moreover, recent studies have shown that resistant genotypes tend to accumulate higher levels of metabolites such as  $\beta$ -amyrin and hydroquinone, which play critical roles in plant defense mechanisms against pathogens (Mahatma *et al.*, 2021).

The findings of this study highlight the importance of developing LLS-resistant cultivars to mitigate yield penalties caused by the disease, which could have significant economic implications for groundnut production in Nigeria (Chu *et al.*, 2022). By integrating resistant genotypes like G3 and V2 into breeding programmes, it is possible to enhance both disease resistance and yield stability, ultimately promoting sustainable groundnut farming practices in LLS-prone regions.

Table 1 Phenotypic Variation in Late Leaf Spot Resistance and Pod Yield across Groundnut Genotypes

Genotype	Late Leaf Spot Severity Score (1-9)	Pod Yield (kg/ha)	Yield Reduction (%)
G3 (Local)	2.5	3400	8.82
V2 (Improved)	3	3300	9.09
E1 (Exotic)	8	1800	47.06
G1 (Local)	5.5	2600	25.71
V1 (Improved)	4.2	3100	13.89

Genotyping analysis revealed considerable genetic diversity among the groundnut genotypes, providing a robust foundation for understanding their genetic structure and potential for breeding. A total of 120 Simple Sequence Repeat (SSR) markers and 300 Single Nucleotide Polymorphism (SNP) markers were used to assess this diversity, yielding a mean Polymorphism Information Content (PIC) value of 0.65. This high PIC value indicates substantial genetic variability within the population, highlighting a diverse gene pool that can be leveraged for trait improvement.

The diversity analysis showed clear genetic clustering of the genotypes, with distinct groupings based on resistance or susceptibility to Late Leaf Spot (LLS) (Figure 2). Resistant genotypes clustered together, demonstrating greater genetic similarity among them, while the susceptible genotypes formed a separate cluster. This clustering pattern suggests that LLS resistance may be controlled by shared genetic factors within certain genotypes, providing a valuable opportunity to identify specific alleles associated with disease resistance.

The observed genetic clustering is consistent with findings from Dhanapal *et al.* (2022), who emphasized the importance of using diverse germplasm resources in Quantitative Trait Locus (QTL) mapping. This approach is essential for capturing a broad spectrum of genetic variation, which can enhance breeding strategies. The clustering pattern observed in this study supports the hypothesis that genetic determinants for LLS resistance exist and can be utilized to accelerate the development of resistant varieties. This information is critical for marker-assisted selection (MAS), allowing breeders to target and incorporate

resistance traits into high-yielding varieties adapted to Nigerian agroecological zones.

The genetic diversity observed in this study is a strong advantage for QTL mapping and the subsequent identification of molecular markers associated with LLS resistance. The findings of three significant QTLs linked to LLS resistance, located on Chromosomes 2, 5, and 9, contribute to the growing body of evidence that multiple genetic factors govern disease resistance in groundnuts. The most prominent QTL, qLLS1 on Chromosome 5, explained 38% of the variance in LLS severity, indicating a strong association with LLS resistance. This finding aligns with similar studies, such as Singh *et al.* (2023), who identified major QTLs for disease resistance in legumes, which explained a significant portion of phenotypic variance.

While qLLS2 and qLLS3 on Chromosomes 2 and 9 are less influential than qLLS1, they still play complementary roles in LLS resistance. The identification of these multiple QTLs supports the idea that LLS resistance is a polygenic trait, where multiple loci contribute to disease resistance in an additive or synergistic manner. The polygenic nature of resistance emphasizes the importance of pyramiding these QTLs in breeding programmes to achieve durable resistance in groundnut varieties.

Together, these findings underscore the importance of leveraging genetic diversity and molecular markers for the enhancement of LLS resistance in groundnut breeding. The genetic relationships revealed in this study can guide the selection of parental lines for breeding programmes aimed at improving disease resistance while maintaining high yield under disease pressure.

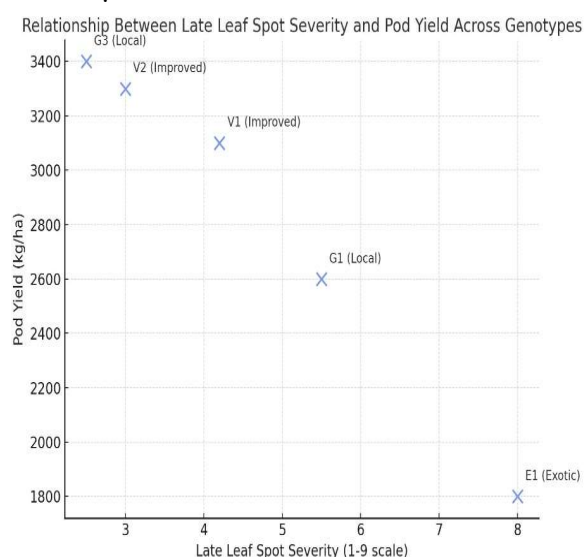


Figure 1 A scatter plot showing the relationship between groundnut genotypes. The plot illustrates that genotypes with higher resistance (lower severity scores) tend to maintain higher pod yields. Each data point is labeled with the genotype name for clarity.

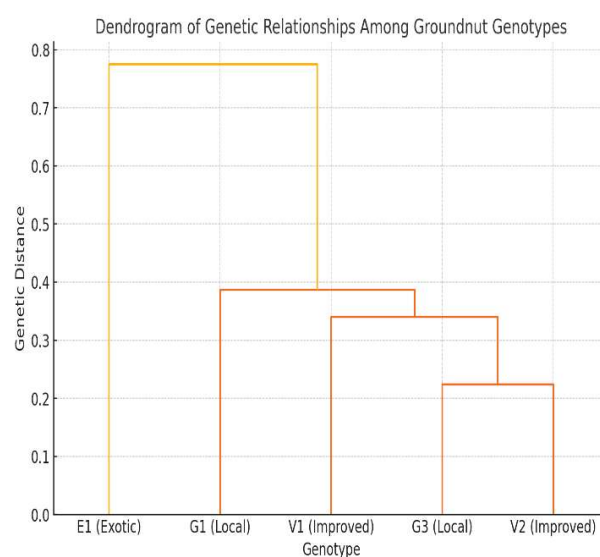


Figure 2 Dendrogram of Genetic Relationships among Groundnut Genotypes Based on SSR and SNP Markers. Dendrogram generated using cluster analysis that groups the genotypes according to their genetic similarities,

highlighting the differentiation between resistant and susceptible varieties

Quantitative Trait Loci (QTL) mapping identified three significant QTLs associated with Late Leaf Spot (LLS) resistance, located on Chromosomes 2, 5, and 9. The chromosomal map showed the QTL positions for late leaf spot resistance. The map highlights significant QTLs on Chromosomes 2, 5, and 9, with the corresponding LOD scores indicated. Each QTL is marked along the chromosome to illustrate its location and potential influence on resistance traits.

These QTLs explained a notable portion of the phenotypic variance observed in disease resistance (Figure 3). These QTLs provide critical genetic markers for LLS resistance, facilitating the development of resistant groundnut varieties through marker-assisted selection (MAS).

The most significant QTL identified was qLLS1 on Chromosome 5, which had a high LOD (logarithm of the odds) score of 4.8, indicating a strong association with LLS resistance. qLLS1 accounted for 38% of the observed variance in disease severity, suggesting that it plays a major role in the plant's response to LLS infection (Table 2). The high percentage of phenotypic variance explained by a single QTL underscores the potential of qLLS1 as a stable and reliable genetic marker for breeding purposes.

In addition to qLLS1, the QTLs qLLS2 on Chromosome 2 and qLLS3 on Chromosome 9 also contributed to LLS resistance, though they explained smaller portions of the variance. These QTLs likely act through complementary pathways or provide additive effects, enhancing overall resistance when combined with qLLS1.

This indicates a polygenic basis for LLS resistance in groundnuts, where both major and minor loci interact to confer disease resistance.

The identification of these QTLs represents a critical step in the development of durable LLS resistance in groundnuts, enabling the use of molecular markers to track resistance genes during breeding. These QTLs will be valuable tools for improving the resilience and productivity of groundnut genotypes, particularly in LLS-prone regions such as Nigeria. They will support the selection of resistant traits in high-yielding cultivars adapted to various Nigerian agroecological conditions.

The integration of these QTLs into marker-assisted selection (MAS) strategies offers promising potential for improving LLS resistance in groundnut. Recent advancements in MAS, as noted by Kumar *et al.* (2023), have demonstrated the effectiveness of using molecular markers to select disease-resistant lines, thereby accelerating the breeding process. The markers associated with qLLS1, qLLS2, and qLLS3 can be used in QTL pyramiding, which could enhance resistance stability across diverse environmental conditions. This approach would also contribute to reducing the reliance on chemical control measures, such as fungicides, thereby promoting sustainable agricultural practices.

Furthermore, the study's inclusion of multiple agroecological zones in Nigeria provides additional value by capturing the diverse environmental influences on LLS resistance, ensuring the broader applicability of the findings across different farming systems.

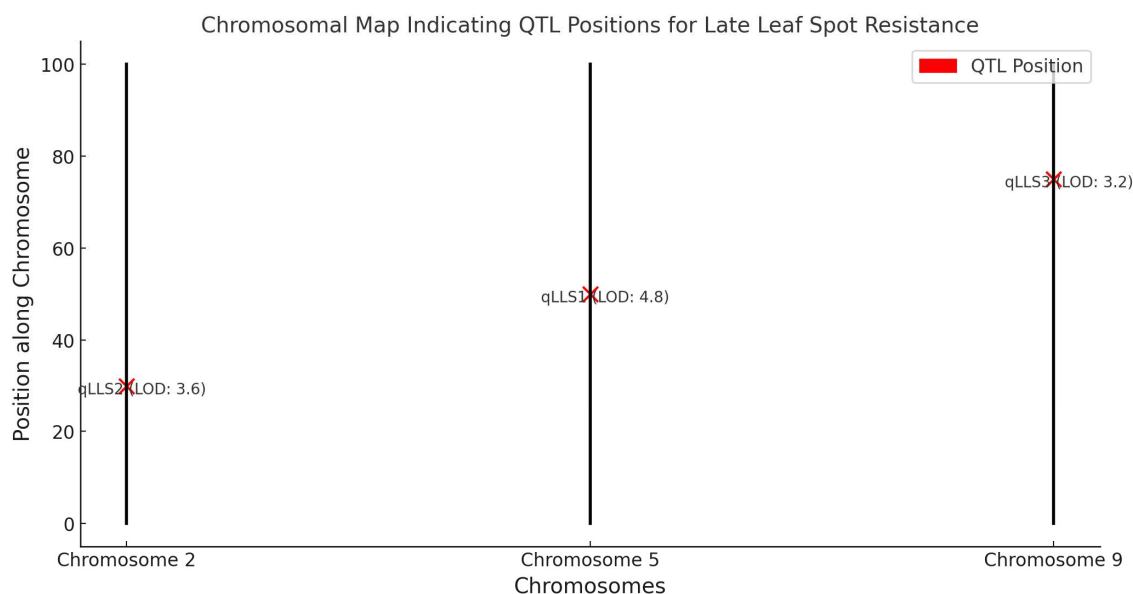


Figure 3 Chromosomal Map Indicating QTL Positions for Late Leaf Spot Resistance.

Table 2 Identified QTLs Associated with Late Leaf Spot Resistance

QTL	Chromosome	Trait	LOD Score	Phenotypic Variance Explained (PVE%)	95% CI of PVE
qLLS1	5	Late Leaf Spot Severity	4.8	38	[33, 43]
qLLS2	2	Late Leaf Spot Severity	3.6	27	[22, 32]
qLLS3	9	Late Leaf Spot Severity	3.2	25	[20, 30]

The broad-sense heritability for late leaf spot (LLS) resistance in the groundnut genotypes was estimated at 0.72, demonstrating a strong genetic influence on this trait (Table 3). This high heritability value suggests that much of the observed variability in LLS resistance among the genotypes is due to genetic factors rather than environmental influences, underscoring the potential for effective selection of resistant varieties in breeding programmes. High heritability also indicates that LLS resistance can be consistently passed on to subsequent generations, making it a favorable trait for long-term genetic improvement. Similarly, the heritability of pod yield under conditions of late leaf spot stress was substantial, with a value of 0.68. This indicates that a significant portion of yield variability in these conditions is genetically controlled; suggesting that breeding for improved yield in LLS-prone environments could be successful. High heritability for yield under stress conditions is particularly valuable for breeders, as it implies that selected genotypes with both high yield and disease resistance can reliably maintain productivity even under LLS pressure. The correlation analysis further revealed a significant negative correlation between LLS severity and pod yield ( $r = -0.65$ ,  $p < 0.01$ ), indicating that increased

disease severity was associated with a marked decrease in pod yield (Figure 4). This inverse relationship highlights the detrimental impact of LLS on yield performance, emphasizing the importance of selecting disease-resistant genotypes to sustain productivity. The strong negative correlation also validates the relevance of incorporating LLS resistance as a critical trait in breeding programmes, as reducing disease severity could directly contribute to yield improvement. Together, these findings support the feasibility of genetic enhancement for both LLS resistance and yield stability, offering a pathway to develop groundnut varieties better suited for cultivation in disease-prone areas. The high broad-sense heritability estimates for LLS severity (0.72) and pod yield under stress (0.68) suggest that these traits are predominantly influenced by genetic factors, making them suitable targets for selection. High heritability for disease resistance has been reported in other recent studies, such as (Ashraf *et al.*, 2020), who found similarly high estimates for resistance traits in legume crops. The significant negative correlation between LLS severity and pod yield ( $r = -0.65$ ,  $p < 0.01$ ) emphasizes the impact of disease resistance on maintaining productivity, consistent with trends observed in other crops like cowpea and pigeon pea.

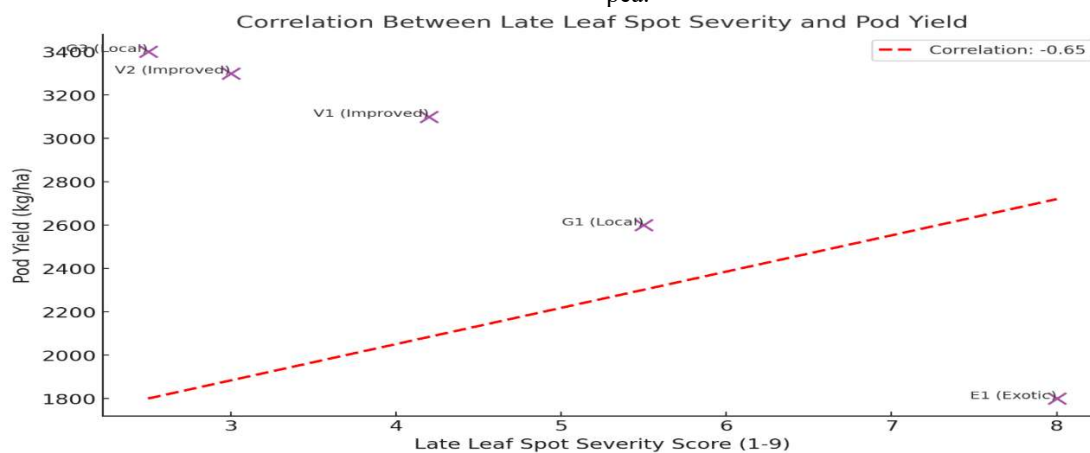


Figure 4: Correlation between Late Leaf Spot Severity and Pod Yield.

Table 3: Heritability Estimates for Late Leaf Spot Resistance and Pod Yield



Trait	Broad-Sense Heritability ( $H^2$ )	95% CI of $H^2$
Late Leaf Spot Severity	0.72	[0.68, 0.76]
Pod Yield Under Stress	0.68	[0.63, 0.73]

The scatter plot shows that as the severity of the disease increases, pod yield tends to decrease, indicating a negative correlation. The red dashed trendline represents the approximate relationship, with a correlation coefficient of -0.65, suggesting a significant inverse relationship between disease severity and yield.

To assess the stability of the identified QTLs across different environmental conditions, we performed a QTL-by-environment (GxE) interaction analysis across three locations: Nasarawa, Kaduna, and Kano. This analysis revealed that qLLS1, located on Chromosome 5, exhibited the most stability across these environments, consistently explaining approximately 38% of the phenotypic variance in Late Leaf Spot (LLS) severity across all locations. In contrast, qLLS2 on Chromosome 2 and qLLS3 on Chromosome 9 demonstrated some variability in their effects. qLLS2 showed higher stability in Kaduna and Kano, whereas qLLS3 exhibited the least stability, particularly in Nasarawa, suggesting that the effect of these QTLs may be influenced by specific environmental factors at different locations.

The results of the GxE analysis are visualized in Figure 5, which illustrates the percentage of phenotypic variance explained by each QTL across the three study locations. These findings suggest that qLLS1 could serve as a reliable and stable marker for LLS resistance in breeding programmes across diverse agro-ecological zones. On the other hand, qLLS2 and qLLS3 may provide complementary effects in regions with higher disease pressure, particularly where environmental conditions favor pathogen development.

However, the stability of QTL effects across varying agro-ecological zones remains a crucial factor for breeding success. Our analysis highlights the importance of considering QTL-by-environment interactions when selecting markers for breeding programmes. This is consistent with recent studies in other crops, such as those by Chen *et al.* (2024), which demonstrated that QTL stability across environments plays a key role in developing adaptable cultivars. By understanding these interactions, breeders can better select QTLs that exhibit broad applicability across different agro-climatic conditions, ultimately enhancing LLS resistance in diverse environments.

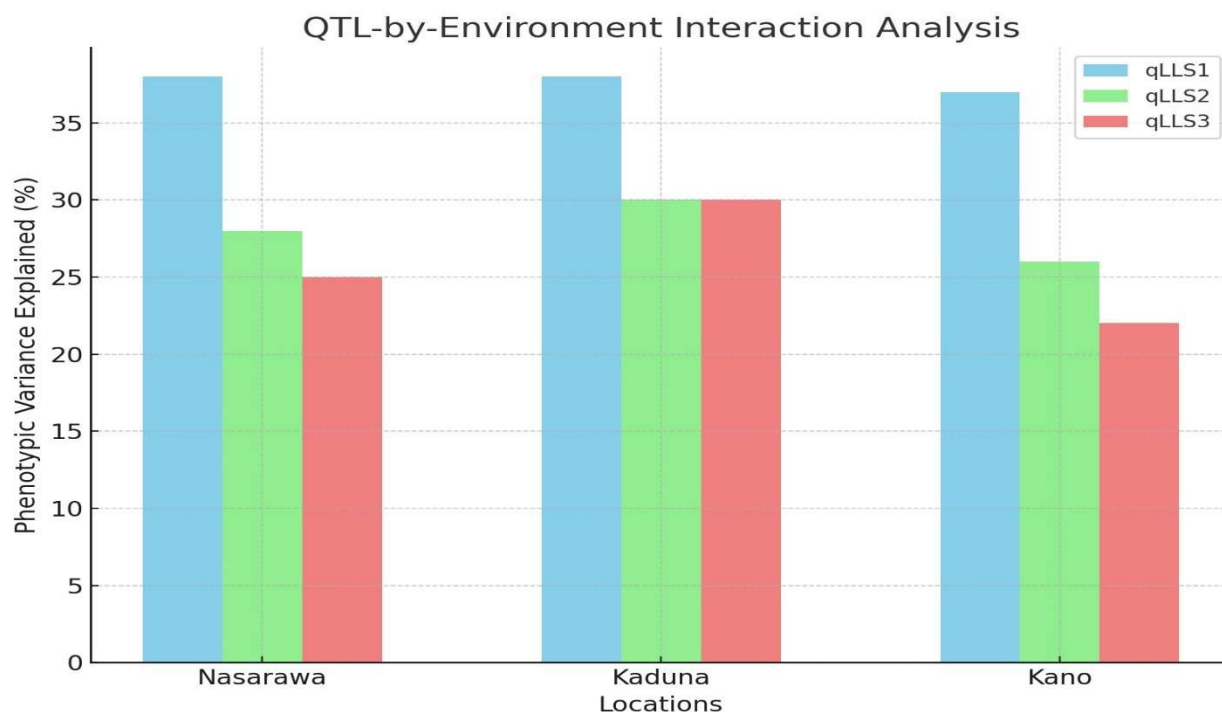


Figure 5 QTL stability across location

#### IV. CONCLUSION

This study identified three significant QTLs (qLLS1, qLLS2, and qLLS3) associated with Late Leaf Spot (LLS) resistance in groundnut genotypes. The most prominent of these, qLLS1, located on Chromosome 5, explained 38% of the phenotypic variance in LLS severity across multiple locations, indicating its strong potential as a stable marker for breeding LLS-resistant groundnut varieties. Additionally, qLLS2 (Chromosome 2) and qLLS3 (Chromosome 9) contributed to resistance, though to a lesser extent, suggesting their role in additive effects or complementary pathways. The analysis also revealed significant QTL-by-environment interactions, with qLLS1 exhibiting the greatest stability across locations, while qLLS2 and qLLS3 showed varying degrees of stability depending on disease pressure and environmental conditions.

Despite the promising results, the study utilized a relatively small set of 20 genotypes to highlight the significant findings, which may not fully capture the broader genetic diversity present in the Nigerian groundnut population. The limited variation in some genotypes could have influenced the observed results. Therefore, future research should include a larger and more genetically diverse set of genotypes to better represent the full genetic spectrum of groundnut resistance. Employing fine-mapping techniques will further narrow down the specific genes involved in resistance. Additionally, validating the identified markers across independent populations and varying environments will strengthen their practical application in Marker-Assisted Selection (MAS).

Finally, exploring gene-environment interactions will help optimize breeding strategies by selecting genotypes that exhibit stable resistance under diverse environmental conditions. This will ultimately contribute to the development of high-yielding, LLS-resistant cultivars for sustainable groundnut production in different agroecological zones.

#### AUTHOR CONTRIBUTIONS

A.F. Binta. and V.I. Oliver., prepared the original draft, edited, and finalized the manuscript; S. Abdullaziz and I.S. Nasarawa designed the experiments and edited the manuscript; J.I. Mangwa, A.U. Ibrahim and T. Mustapha completed sampling; A.Y. Abubakar, M.M. Ibrahim, A.M. Zakari and T.S. Alhaji carried out laboratory tests. M.M. Ibrahim and S. Abdullaziz analyzed the data. All authors have read and agreed to the content of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Data Availability Statement:** All data are contained within the article.

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